Please add the following new claim:

39. A method of making a chimeric mouse, comprising:

a. creating an immunetolerant mouse, said immunetolerant mouse having a degenerated liver due to the presence of a secreted urokinase-type plasminogen activator (uPA) and lacking functional T and B cells; and

b. transplanting human hepatocytes having at least 80% viability by intrasplenic injection to repopulate the parenchyma of the degenerated liver.

40. The method of claim 39 wherein said immunetolerant mouse is about 10-14 days old at the time of transplanting said human hepatocytes.

41. The method of claim 40 wherein the transplanted human hepatocytes reconstitute approximately 10% of the degenerated liver.

REMARKS

Reconsideration of this application is respectfully requested in view of the above amendments and the following remarks.

I. Claim Status. Claims 1, 8, 15, 25, 37 and 38 have been amended without prejudice or disclaimer. Support for immunetolerant mice lacking functional B and T cells is found throughout the specification, e.g., in the Abstract, line 2 and at page 11, lines 1-2. Support for liver degeneration due to the presence of the secreted urokinase-type plasminogen activator (uPA) is found throughout the specification, e.g., at page 5, lines 31-23. The wild-type secreted form of uPA was used in all of Examples of the application. Support for repopulation of the immunetolerant uPA mouse with xenogenic mammalian hepatocytes that are infected with at least one compatible mammalian hepatitis virus capable of causing hepatocellular carcinoma in said xenogenic hepatocytes be found in the specification at page 13, lines 5-28.

Claims 39-41 have been added. Support for the added claims is found in the specification at page 29, line 25 through page 30, line 6.

By this Amendment claims 1-41 are pending in the application.

II. Added claims. Claim 39 is directed to a method for creating an immunetolerant mouse with degenerated liver repopulated with human hepatocytes, obtained by effecting liver degeneration with secreted uPA and transplanting human hepatocytes having at least 80% viability via intrasplenic injection. Claim 40 is directed further to this method wherein the transplantation is performed when the mice are about 10-14 days. Claim 41 is directed still further to the case where the transplanted human hepatocytes reconstitute approximately 10% of the liver. The prior art fails to disclose or suggest the method of claims 39-41. Allowance of these claims is respectfully requested.

III. Claim rejections.

(i) Rejections under 35 U.S.C. § 112, first paragraph. Claims 1-38 have been rejected for lack of enablement as the specification allegedly does not enable claims drawn to immunetolerant mice deficient in T and B cells. In response, as suggested by the Examiner, the claims have been amended to be directed to immunetolerant mice lacking functional T and B cells. Hence, this rejection is believed to have been addressed and overcome.

The Examiner also alleges that the specification fails to support all methods for creating a degenerated liver. In response, the claims have been amended to be drawn to a chimeric immunetolerant mouse with liver degenerated due to presence of a secreted urokinase-type plasminogen activator (uPA). This rejection is believed to have been addressed and overcome.

Claims 25-35 have been rejected because the Examiner alleges that, because not all mammalian hepatitis virus viruses result in HCC, it is unclear how potential anti-cancer agents may be screened by administration prior to onset of HCC. In response, claims 25-35 have been amended to be directed to a test system wherein the xenogenic hepatocytes are infected with a compatible mammalian hepatitis virus capable of causing hepatocellular carcinoma in said xenogenic hepatocytes. It is submitted that it would be readily apparent to one of ordinary skill in the art that the instantly claimed methods could be used to screen potential anti-cancer agents by administering the potential anti-cancer agent to experimental and control animals prior to onset of HCC and then comparing the observed rate of HCC development in the experimental and control animals. Hence, contrary to the Examiner's assertion, amended claims 25-35 are operable for screening of potential anti-cancer agents.

In view of the amendments to the claims and for the reasons set forth above, Applicants respectfully submit that all rejections of claims 1-38 under 35 U.S.C. § 112, first paragraph have been addressed and overcome. Accordingly, Applicants respectfully request reconsideration of claims 1-38 and withdrawal of all rejections under 35 U.S.C. § 112, first paragraph.

(ii) Rejections under 35 U.S.C. § 102(e). Claims 1-5, 8-12, 15-21, 25-33 and 36-38 have been rejected as allegedly anticipated by Kay et al., U.S. Patent No. 5,980,886 ("the '886 Patent"). The claims have been amended to be directed to be drawn to a chimeric immunetolerant mouse with liver degenerated due to presence of a secreted urokinase-type plasminogen activator (uPA). The '886 Patent, in contrast, discloses only that "[s]imultaneous with or subsequent to expression of the secretion impaired uPA transgene, non-native (e.g., human) hepatocytes are implanted in the transgenic animal..." Hence, the '886 Patent fails to

disclose or suggest that human hepatocytes should be implanted into an immunetolerant mouse with liver degenerated by the presence of secreted uPA, as defined in the present claims. Accordingly, the '886 Patent does not anticipate the amended claims.

To be available as a reference against the present claims, the '886 Patent must meet all requirements of 35 U.S.C. § 112, first paragraph. Accordingly, Applicants continue to assert that the '886 Patent does not comply with 35 U.S.C. § 112, first paragraph and is therefore not a proper reference against the present claims, because it fails to enable one of ordinary skill in the field of the application to make the disclosed immunodeficient mouse expressing a secretion-impaired uPA transgene reconstituted with human hepatocytes without undue experimentation. (*In re Wands*, 858 F.2d 731, 737 ("[E]xperimentation needed to practice the invention must not be undue.")

The Examiner continues to assert that the '886 Patent enables one of ordinary skill to create the disclosed immunodeficient mouse expressing a secretion-impaired uPA transgene reconstituted with human hepatocytes. Applicants disagree. The '866 Patent fails to provide any working examples for such a mouse. As set forth in Applicants' response to the previous Office Action, the '866 Patent fails to give specific guidance on how to create the an immunodeficient mouse expressing a secretion-impaired uPA transgene reconstituted with human hepatocytes. The '886 Patent offers no guidance, e.g, as to: (i) what type of human hepatocytes (e.g., primary cells or cultured cells) are to be transplanted, (ii) the number of cells to be transplanted, (iii) the age or specific genotype of the immunodeficient mouse, (iv) the conditions that should be used to cause liver degeneration in an immunetolerant mouse or what percentage of the liver is, or should be, degenerated prior to transplantation; and (v) what level or over what period of time the modified, non-secreted uPA transgene disclosed in Kay should be

expressed in the immunetolerant mouse to cause liver degeneration prior to transplantation of human hepatocytes.

In one particular instance, the Examiner asserts that the '886 Patent teaches that a transgene encoding a modified uPA transgene is not secreted by hepatocytes into the bloodstream, but otherwise functions the same as wild-type uPA. The '886 Patent, and hence also the Examiner, however, fails to point to any evidence in the '886 Patent that supports this conclusion. Nor does the Examiner provide any additional references to support this statement.

The Examiner asserts generally that specific details lacking in the '886 Patent are within the skill level of one of ordinary skill in the art. As of the priority date of the '886 Patent, however, the state of the art, as represented by the references cited in the '886 Patent (Rhim et al. *Science* 263:1149-1152, 1994, already of record in the application; and WO 94/02601, enclosed with accompanying Information Disclosure Statement), was such that only native (i.e., mouse) hepatocytes had been used successfully to repopulate mouse liver degenerated with the secreted form of uPA. Hence the Examiner has no basis for asserting that details lacking in the '886 Patent could be provided by one of ordinary skill in the art.

In support of their position that the '886 Patent is non-enabling, Applicants enclose a Declaration from co-inventor Charles E. Rogler. The Declaration states that Dr. Rogler spoke with Dr. Mark A. Kay, co-inventor of the '886 Patent on two separate occasions. As set forth in the Declaration, on both occasions Dr. Kay stated to Dr. Rogler that his laboratory had attempted to make the chimeric immunetolerant mouse with degenerated liver repopulated with human hepatocytes that is described in the '886 Patent, but that these attempts had failed. Hence, the disclosure of the '886 Patent failed to enable even a laboratory highly skilled in liver degeneration and reconstitution techniques to practice the disclosed invention.

Finally, Applicants note that, as set forth in the Rogler Declaration, other than the work of the present inventors, the first account of a chimeric immunetolerant mouse with degenerated liver repopulated with human hepatocytes were published at least over six years after the priority date of the '886 Patent. Applicants note that this mouse-human chimeric animal was obtained using the secreted form of uPA to effect liver degeneration. Hence, Applicants are not aware of any successful attempt to create an immunetolerant mouse with liver degenerated with non-secreted uPA and repopulated with human hepatocytes.

For the reasons set forth above, Applicants submit that claims 1-5, 8-12, 15-21, 25-33 and 36-38 as amended are not anticipated by the '886 Patent. Accordingly, Applicants respectfully request reconsideration of claims 1-5, 8-12, 15-21, 25-33 and 36-38 and withdrawal of all rejections of these claims under 35 U.S.C. § 102 (e).

(ii) *Rejections under 35 U.S.C. § 103(a).* Claims 1-38 have been rejected as allegedly obvious over Kay et al., U.S. Patent 5,980,886, Alt et al., U.S. Patent 5,583,278 and Roggendorf et al., *Intervirology* 38:100-112, 1995.

Applicants respectfully traverse the rejections on the grounds that the cited references, neither alone nor in combination, render the claims obvious. The claims have been amended to be directed to a chimeric immunetolerant mouse with liver degenerated due to the presence of a secreted urokinase-type plasminogen activator (uPA). As set forth above, in the discussion of the rejections under rule 102(e), the '886 Patent, discloses only that "[s]imultaneous with or subsequent to expression of the secretion impaired uPA transgene, non-native (e.g., human) hepatocytes are implanted in the transgenic animal..." The '886 Patent fails to make any specific suggestion or disclose any benefit to be derived by substituting the

secreted uPA for the non-secreted uPA in the disclosed method. Accordingly, the present claims are not obvious over the '886 Patent.

Nor is the defect in the '886 Patent cured by the other cited references. The '278 Patent is concerned solely with construction of mice deficient in recombination activating genes (RAG genes) with improved SCID phenotypes. Roggendorf et al. is a general discussion of the woodchuck model for studying hepatitis B virus infection in man. Neither of the references include any disclosure or suggestion regarding uPA-induced liver degeneration. Hence, neither the '278 Patent nor Roggendorf et al., either alone or combination with the '886 Patent, discloses or suggests any benefit to using the secreted uPA transgene to effect liver degeneration in an immunetolerant mouse for use as an acceptor for donor human hepatocytes.

Lastly, as described above in the discussion of the rejections under rule 102(b), Applicants have asserted that the '886 Patent, cited as the primary reference for the present obviousness rejection, is not enabling for the disclosed immunodeficient mouse expressing a secretion-impaired uPA transgene reconstituted with human hepatocytes. Neither do the '278 Patent nor Roggendorf et al. provide any disclosure that would enable one of ordinary skill in the art to create such a mouse. Hence, the combination of the '886 and '278 Patents and Roggendorf et al. also fails to enable the mouse disclosed in the '886 Patent.

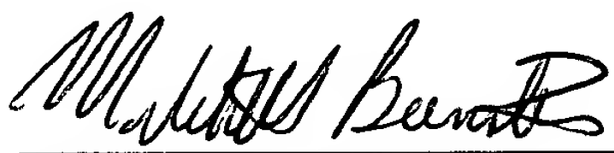
For the reasons set forth above, Applicants submit that claims 1-38 as amended are not obvious over the '886 Patent in view of the '278 Patent and Roggendorf et al. Accordingly, Applicants respectfully request reconsideration of claims 1-38 and withdrawal of all rejections of these claims under 35 U.S.C. § 103 (a).

CONCLUSION

Therefore, in view of the above amendments and remarks, reconsideration of this application is respectfully requested It is earnestly solicited that all pending claims be allowed and the case passed to issue.

If there are any other issues remaining which the Examiner believes could be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

Respectfully submitted,



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PATENT TRADEMARK OFFICE

Docket No.: 3361/1D888-US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Charles E. ROGLER et al.

Serial No.: 09/344,189 Art Unit: 1632

Filed: June 24, 1999 Examiner: P. Paras, Jr.

For: **CHRONIC HEPATITIS VIRUS INFECTION AND CLONAL HEPATOCELLULAR CARCINOMA IN MOUSE REPOPULATED LIVERS**

MARK-UP TO RESPONSE UNDER 37 C.F.R. § 1.111

The accompanying Amendment amends the subject application as follows:

IN THE CLAIMS

Claims 39-41 have been added.

Claims 1, 8, 15, 25, 37 and 38 have been amended as follows:

1. (Thrice amended) A method of making a chimeric mouse, comprising:

a. creating an immunetolerant mouse which has a degenerated liver due to the presence of a secreted urokinase-type plasminogen activator (uPA) and which is [deficient in] lacking functional T and B cells; and

b. transplanting xenogenic mammalian hepatocytes to repopulate the parenchyma of the degenerated liver, said xenogenic mammalian hepatocytes being infected with at least one compatible mammalian hepatitis virus.

8. (Thrice amended) A chimeric mouse model system for hepatitis comprising an immunetolerant mouse [deficient in] lacking functional T and B cells having a degenerated liver parenchyma due to presence of a secreted urokinase-type plasminogen activator (uPA) that is repopulated with transplanted xenogenic mammalian hepatocytes, said xenogenic mammalian hepatocytes infected with a compatible mammalian hepatitis virus.

15. (Twice Amended) A method for screening a test compound for anti-viral activity, comprising:

a. administering said test compound to an immunetolerant chimeric mouse [deficient in] lacking functional T and B cells which has a degenerated liver parenchyma due to presence of a secreted urokinase-type plasminogen activator (uPA) that is repopulated with transplanted xenogenic mammalian hepatocytes and wherein the xenogenic hepatocytes are infected with at least one compatible mammalian hepatitis virus; and

b. assaying the level of replication of the virus.

25. (Twice amended) A method for screening a test compound for anti-cancer activity, comprising:

a. administering said test compound to [an] immunetolerant chimeric mice [mouse deficient in] lacking functional T and B cells which [has] have degenerated liver parenchyma due to presence of a secreted urokinase-type plasminogen activator (uPA) that is repopulated with transplanted xenogenic mammalian hepatocytes and wherein the xenogenic

mammalian hepatocytes are infected with at least one compatible mammalian hepatitis virus capable of causing hepatocellular carcinoma in said xenogenic hepatocytes; and

b. assaying [the] said mice for the development of hepatocellular carcinoma [in said mice].

37. (Amended) A method of making a chimeric mouse, comprising:

a. creating an immunetolerant mouse, said immunetolerant mouse having a degenerated liver due to the presence of a secreted urokinase-type plasminogen activator (uPA) and [being deficient in] lacking functional T and B cells; and

b. repopulating the parenchyma of the degenerated liver by transplanting xenogenic mammalian hepatocytes that are a natural host for infection with one or more compatible hepatitis virus into said liver.

38. (Amended) A chimeric mouse model system for hepatitis comprising an immunetolerant mouse deficient in T and B cells having a degenerated liver parenchyma due to the presence of a secreted urokinase-type plasminogen activator (uPA) that is repopulated with transplanted xenogenic mammalian hepatocytes that are a natural host for infection with one or more compatible hepatitis virus.